

Active and inactive conformations of the epidermal growth factor receptor

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Abstract

The members of the EGFR (epidermal growth factor receptor) family of RTKs (receptor tyrosine kinases), also known as the ErbB or HER family, have been implicated in many human cancers. Structural studies of the EGFR extracellular region (sEGFR) have led to the proposal of a novel mechanism for ligand-induced receptor dimerization. In this model EGF binding induces a dramatic conformational change in EGFR, exposing a dimerization site that is normally occluded in the inactivated conformation, and thus promoting the formation of an entirely receptor-mediated dimer. It is well established that antibodies against the extracellular region of EGFR that prevent ligand binding and/or receptor signalling can inhibit tumour growth *in vivo*. At least five such anti-EGFR antibodies are currently in clinical trials and one, C225/cetuximab (ErbixTM), was recently approved in the U.S. and Europe for use in advanced colorectal cancers. Recent structural studies of ErbB2 in complex with anti-ErbB2 antibodies (trastuzumab/HerceptinTM and pertuzumab/OmnitargTM) have provided significant insights into how these drugs function. There have been no such studies for similar EGFR-targeted drugs to date. The implications of this model for the possible mechanisms of antibody-mediated inhibition of EGFR are discussed.

Mechanism of EGFR dimerization

Recent structural data have led to the proposal of a novel model for the ligand-induced dimerization and activation of the EGFR (epidermal growth factor receptor) family of RTKs (receptor tyrosine kinases) (Figure 1) [1–4]. Growth factor binding to the extracellular domain of EGFR induces dimerization that is mediated entirely by direct receptor–receptor contacts (Figure 1B) [1,2]. This is in stark contrast with the situation seen for almost every other growth factor receptor studied, in which dimerization is instead mediated by one or more bivalent ligand molecules [5,6]. The monomeric growth factor ligands of EGFR, such as EGF and TGF- α (transforming growth factor- α), simultaneously contact two of the four subdomains (domains I and III) in the extracellular region of EGFR (sEGFR; Figure 1B). Thus EGF and TGF α are bivalent, like other growth factors, but contact two binding sites within a single receptor molecule rather than spanning the dimerization interface and linking the two receptor molecules. Growth factor binding to domains I and III of sEGFR imposes constraints on the domain arrangement within sEGFR, and stabilizes the conformation required for dimerization. The most notable consequence of ligand binding is to ‘expose’ a loop in domain II that is involved in an intramolecular interaction with domain IV in the inactive sEGFR configuration (Figure 1A) [3], but extends across the dimer interface in Figure 1(B) to

comprise the primary dimerization site or ‘dimerization arm’ [2]. The monomeric, tethered conformation, also seen for the unliganded extracellular region of ErbB3 [7], holds domains I and III too distant from one another for the small monomeric growth factor agonist to bind both domains simultaneously. Only by undergoing a major conformational rearrangement that releases the dimerization arm from its intramolecular tether and ‘extends’ the molecule to the configuration seen in Figure 1(B) can ligand bind with high affinity.

The physical transformation required to take sEGFR from the tethered to the extended conformation can be modelled on the basis of the crystallographic data [3]. However, these data give no information as to precisely how the conformational change is induced. There are (at least) two possible mechanisms by which ligand binding could promote the conformational rearrangement seen in sEGFR and lead to receptor dimerization and activation. In one possible mechanism ligand binding to domain I or domain III may trigger a conformational change that in turn ‘releases’ the dimerization arm from a fixed intramolecular tether, allowing the molecule to adopt the extended conformation and dimerize. We consider this mechanism unlikely. Indeed, as shown in Figure 2, there is essentially no discernible change in the conformation of domain III of sEGFR when comparing structures determined with no ligand (grey), EGF (yellow) or TGF α (blue) bound. The pairwise r.m.s.d.s (root-mean-square deviations) for main chain atoms are in the range of 0.8 Å (1 Å = 0.1 nm), with the differences between the two ligand-bound domain III structures being no greater than those between ligand-bound and unliganded structures. This similarity even extends to the positions of the side chains that are directly involved in ligand binding, as seen in Figure 2(C).

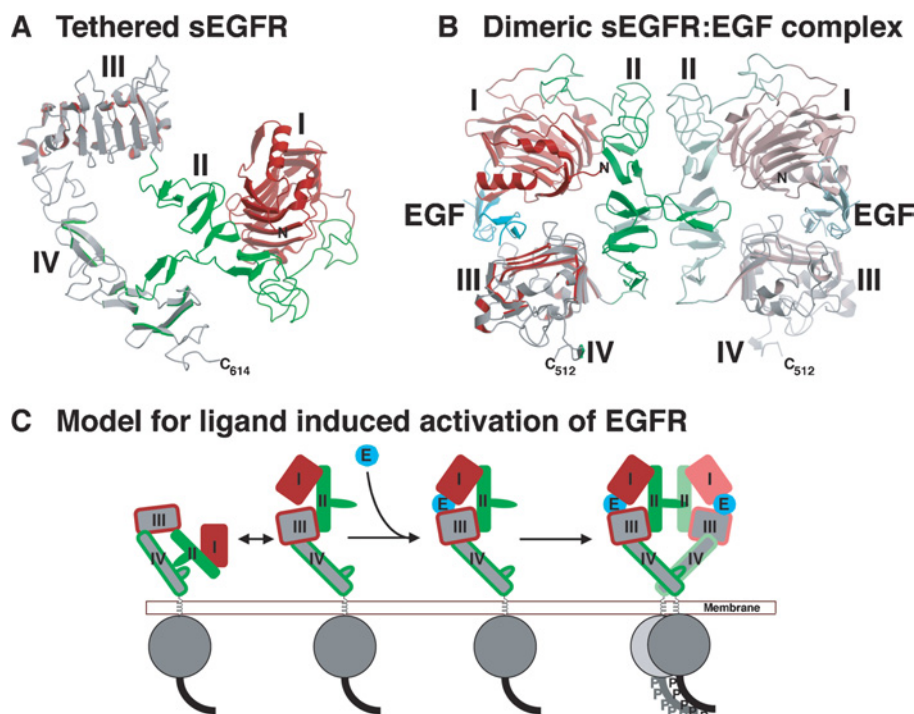
Key words: ErbB receptor, extracellular domain, growth factor, mechanism-based inhibitor, protein structure, target-specific drug.

Abbreviations used: EGF(R), epidermal growth factor (receptor); sEGFR, extracellular region of EGFR; r.m.s.d., root-mean-square deviation; RTK, receptor tyrosine kinase; TGF α , transforming growth factor- α .

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Figure 1 | Conformations of the extracellular region of EGFR

(A) The tethered conformation of sEGFR (PDB identity no. 1NQL). Domain I is shown in red, domain II in green, domains III and IV are in grey, and the sides of the strands and helices are in red and green respectively. (B) The sEGFR:EGF complex (PDB identity no. 1IVO) shown with the same domain colours as in (A), and with EGF in cyan. Colours on the right-hand molecules have been whitened to allow differentiation of domain II. Most of domain IV is absent in this model. (C) Model for ligand-induced activation of EGFR; see the text for further details.



With such small changes it seems highly unlikely that ligand binding to domain III could be ‘sensed’ at the domain II/IV interface, and that this could be responsible for the disruption of this interaction.

A second possibility is that sEGFR is a dynamic structure that explores a wide range of different conformations, and that EGF binding simply ‘traps’ the molecule in a (relatively rare) dimerization-competent configuration, with the domain II dimerization arm exposed. Several observations suggest that this model is more accurate. We estimate that the domain II/IV tether is quite weak, with an interaction energy of just 1–2 kcal/mol. This estimate is based upon the fact that disruption of the intramolecular tether by deletion of domain IV [8] or by mutating key amino acids in domain IV that participate in the tether [3] increases the EGF-binding affinity of sEGFR by 5–30-fold. We take this as an estimate of the energetic barrier to juxtaposing domains I and III ideally for bivalent EGF binding, and thus an estimate of the strength of the autoinhibitory tether. With a ΔG of 1–2 kcal/mol, approx. 85–97% of the intact sEGFR would be expected to be in a tethered configuration at equilibrium, while the remaining 3–15% would be unconstrained and capable of adopting the high-affinity extended form. Thus, assuming that there is no other energetic or kinetic barrier to domain rearrangement, it seems reasonable to propose that EGF binding will select/

trap a dimerization-competent configuration from the unconstrained conformers, thus driving the equilibrium towards receptor dimer.

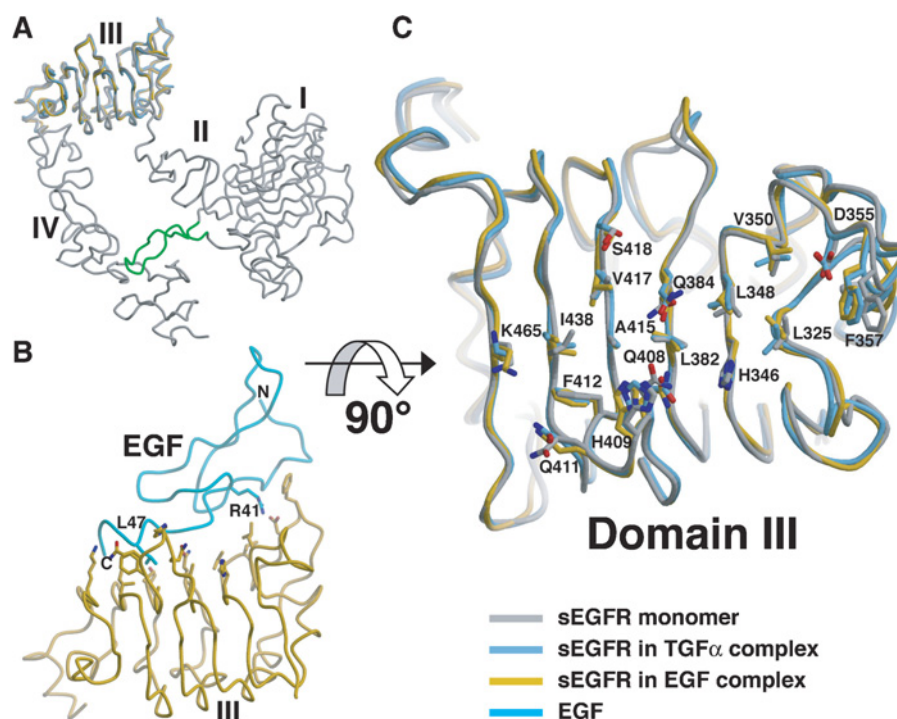
The novel mode of growth factor-induced receptor dimerization seen for EGFR introduces a completely new state of EGFR – the auto-inhibited or tethered state – and suggests that stabilizing this state might represent a new approach for inhibiting EGFR when it is inappropriately activated in human cancer.

Implications for inhibition of EGFR by antibody interactions

The correlation between EGFR and cancer is well established [9]. It was proposed more than 20 years ago that an antibody against the extracellular region of EGFR that was capable of preventing ligand binding and/or receptor signalling could have potential as a cancer therapeutic [10]. At least five such antibodies are currently in clinical trials: IMC-C225/cetuximab/ErbituxTM, EMD 72000, ABX-EGF, ICR62 and h-R3 [11]. The precise epitopes for these clinically relevant ligands are unknown. Although inhibition of EGF binding (and of receptor activation) has been demonstrated in each case, the mechanistic basis for this is not understood. It is not clear whether the effect arises from direct competition

Figure 2 | Comparison of domain III

(A) The tethered structure of sEGFR is shown as a grey 'worm'. The domain III fragments from the sEGFR:EGF complex (yellow) and from the sEGFR:TGF α complex (blue) are superimposed. R.m.s.d.s for main chain atoms between domain III of the tethered sEGFR (PDB identity no. 1NQL) and domain III from the EGF complex is 0.84 Å (PDB identity no. 1IVO, chain A), and with domain III in the TGF α complex is 0.68 Å (PDB identity no. 1MOX, chain A). The r.m.s.d. between the main chain atoms of the two ligand bound domain III structures is 0.67 Å. (B) View of the EGF bound to domain III in the same orientation as in (A). Side chains on domain III that are labels in part (C) are shown, as are Arg-41 and Leu-47 from EGF that make important contacts with domain III. (C) View of the overlay of domain III looking down on to the ligand binding surface (90° rotation about the axis shown by the arrow). The side chains that interact with EGF and TGF α are shown.



for identical binding sites, involves stabilization of particular (autoinhibited) EGFR configurations, or involves other influences.

The simplest possibility for how EGFR-inhibitory antibodies might function is through direct interaction with the EGF-binding site, thus physically blocking the ligand's ability to bind the receptor. Experiments employing interspecies domain-swapping mutants have suggested that the epitopes for several EGFR-inhibitory antibodies (including monoclonal antibodies 425 and 225) are in domain III [12], and may overlap the domain III EGF-binding site. However, it is equally plausible that the antibodies instead reduce EGF-binding affinity indirectly, by stabilizing an EGFR configuration in which the two binding sites on domains I and III cannot simultaneously contact the same bound ligand. For example, these antibodies could stabilize the tethered (monomeric) configuration shown in Figure 1(A) (although many other dimerization-incompetent low-affinity forms can be envisioned).

Finally, structural and other studies with the pertuzumab anti-ErbB2 antibody (Omnitarg/2C4) have elegantly illustrated another route to ErbB receptor inhibition [13].

Pertuzumab binds to the constitutively extended ErbB2 extracellular region [14,15] in a manner that would prevent its dimerization arm from interacting with another ErbB2 molecule (in a homodimer) or another ErbB receptor (in a heterodimer). Pertuzumab efficiently inhibits neuregulin-induced ErbB2/ErbB3 heterodimer formation [13]. A similar approach should also be fruitful in attempts to inhibit EGFR homo- and hetero-dimerization, and structural studies of available inhibitory anti-EGFR antibodies are required to determine whether (as with ErbB2) antibody screening has already exploited this possibility.

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